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REVIEW

Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance

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SUMMARY The genus *Enterobacter* is a member of the ESKAPE group, which contains the major resistant bacterial pathogens. First described in 1960, this group member has proven to be more complex as a result of the exponential evolution of phenotypic and genotypic methods. Today, 22 species belong to the *Enterobacter* genus. These species are described in the environment and have been reported as opportunistic pathogens in plants, animals, and humans. The pathogenicity/virulence of this bacte-

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rium remains rather unclear due to the limited amount of work performed to date in this field. In contrast, its resistance against antibacterial agents has been extensively studied. In the face of antibiotic treatment, it is able to manage different mechanisms of resistance via various local and global regulator genes and the modulation of the expression of different proteins, including enzymes (β-lactamases, etc.) or membrane transporters, such as porins and efflux pumps. During various hospital outbreaks, the *Enterobacter aerogenes* and *E. cloacae* complex exhibited a multidrug-resistant phenotype, which has stimulated questions about the role of cascade regulation in the emergence of these well-adapted clones.

KEYWORDS β-lactamases, *Enterobacter* spp., clinical aspects, diagnosis, efflux, epidemiology, impermeability, multidrug resistance, pathogenicity

INTRODUCTION

The genus *Enterobacter* includes facultative anaerobic Gram-negative bacilli that are 2 mm long, are motile by means of peritrichous flagella, and belong to the family *Enterobacteriaceae*. It was first described in 1960, but changes in taxonomy have occurred in the last 50 years (1). For example, *E. sakazakii* has been reassigned to a new genus, *Cronobacter* (2).

To date, 22 species have been found in the genus *Enterobacter*: *E. aerogenes*, *E. amnigenus*, *E. arachidis*, *E. asburiae*, *E. carcinogenus*, *E. cloacae*, *E. cowanii*, *E. dissolvans*, *E. gergoviae*, *E. helveticus*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. mori*, *E. nimipressuralis*, *E. oryzae*, *E. pulveris*, *E. pyrinus*, *E. radicincitans*, *E. soli*, *E. taylorae*, and *E. turicensis*. Among these species, seven are grouped within the *Enterobacter cloacae* complex group: *E. cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. mori*, and *E. nimipressuralis*. This nomenclature is based on the sharing of phenotypic and especially genotypic characteristics, determined by whole-genome DNA-DNA hybridizations. Indeed, *E. cloacae* shares at least 60% similarity in its genome with the other six members of this group (3).

The genus *Enterobacter* is associated with a variety of environmental habitats. These bacteria are recovered from soil and water and are endophytic or considered phytopathogens for various plant species (4). Some species are frequently associated with bioprocessing and metabolic engineering approaches (5, 6). Moreover, *Enterobacter* spp. are also natural commensals of the animal and human gut microbiota. Among these bacteria, only certain subspecies/species have been associated with hospital-acquired infections and outbreaks (7–12). *Enterobacter* species are members of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which are described as the leading cause of resistant nosocomial infections (7, 10, 11, 13–20). *Enterobacter aerogenes*, *E. cloacae*, and *E. hormaechei* represent the most frequently isolated species described in clinical infections, especially in immunocompromised patients and those hospitalized in an intensive care unit (ICU), due to the adaptation of these species to antimicrobial agents and their behavior as opportunistic pathogens. Several hospital outbreaks have been reported in Europe since the mid-1990s, and the wide use of extensive broad-spectrum antibiotics has stimulated the spread of resistant clones (21–23). These pathogens are frequently associated with a multidrug resistance (MDR) phenotype, mainly due to their adaptation to the hospital environment and their ability to easily acquire numerous genetic mobile elements containing resistance and virulence genes. These species have an intrinsic resistance to ampicillin, amoxicillin, first-generation cephalosporins, and cefoxitin due to the expression of a constitutive AmpC β-lactamase. Moreover, the production of extended-spectrum β-lactamases has been reported in these bacteria, which make their treatment difficult (24, 25). Antibiotic resistance, regulation of resistance genes and the clinical implications of these situations have been extensively studied (26–31).

The accurate identification of species and subspecies remains a challenge. The development of genome sequencing has rapidly modified the phylogeny of the genus,

particularly that of the *E. cloacae* complex (32–34). Due to use of modern molecular techniques, the genus has undergone modifications in classification, and several species have been transferred to and from this genus. For example, four species first identified as *Enterobacter* have been reclassified to the genus *Kosakonia* (*E. cowanii*, *E. arachidis*, *E. oryzae*, and *E. radicinintans*), *E. intermedium* has been reclassified to the genus *Kluyvera*, and *E. sakazakii* has been reclassified to the genus *Cronobacter* (2, 35, 36). Moreover, the current taxonomic position of *E. aerogenes* is still discussed. Since 1971, the proposition of reclassification in the genus *Klebsiella* as *K. aerogenes*, *K. mobilis*, or *K. aeromobilis* because of its motility remains unsettled (37). The phenotypic differences between *E. aerogenes* and the genus *Klebsiella* include motility and the presence of *ornithine decarboxylase* and the lack of urease activity in *E. aerogenes*. However, results from full-genome sequence analysis confirmed that the closest species to *E. aerogenes* was *K. pneumoniae* (37). Considering the taxonomic requirements, the epithet *mobilis* was discussed (38). Recently, both CLSI and EUCAST (https://clsi.org/media/1974/ast_news_update_jan18.pdf, http://www.eucast.org/eucast_news/news_singleview/?tx_ttnews%5Btt_news%5D=316&cHash=000ba6c5b8c2516c98467ef8fbd8dccc) have included this modification in their documents, which now mention *Enterobacter aerogenes* as *Klebsiella aerogenes*. However, until now, scientific reports only occasionally have used *Klebsiella aerogenes* and usually have used *E. aerogenes*. Multilocus sequence analysis (MLSA) of housekeeping genes, in part, and sequencing of the 16S rRNA have recently allowed the characterization of new *Enterobacter* species isolated from human infections or from plants (39, 40). The total genome sequences of the various *Enterobacter* spp. have allowed reevaluation of the genus phylogeny and better evaluation of the importance of those cases where some species were misidentified as other species by routine identification techniques, as was the case of *E. hormaechei* in the *E. cloacae* complex (41).

TAXONOMY OF THE GENUS

Enterobacter amnigenus

The species *E. amnigenus*, described in 1981 by Izard et al., is a rarely isolated bacterium (42). It was suggested by Brady et al. that it be reclassified in the genus *Lelliottia* based on multilocus sequence analysis; however, that change has never been validly published, and *E. amnigenus* still remains the official nomenclature (35). It comprises two genotypically and phenotypically different groups that have been called biogroup 1 and biogroup 2 by the CDC. The strains are generally *ornithine decarboxylase* (ODC) positive and lysine decarboxylase (LDC) and urease negative and ferment melibiose. Strains of *E. amnigenus* biogroup 1 ferment sucrose and raffinose but not D-sorbitol. They are arginine dihydrolase (ADH) negative. Strains of the *E. amnigenus* biogroup 2 ferment D-sorbitol but not sucrose and raffinose. Additional identifying characteristics are presented in Table 1. Strains of *E. amnigenus* are generally susceptible to piperacillin, imipenem, gentamicin, tobramycin, amikacin, nalidixic acid, norfloxacin, ciprofloxacin, and colistin and are resistant to ampicillin, amoxicillin-clavulanic acid, ticarcillin, and cephalothin. Resistance is variable across strains for second- and third-generation cephalosporins, moxalactam, doxycycline, chloramphenicol, and cotrimoxazole (43–45).

Enterobacter cancerogenus

Formerly known as *Erwinia cancerogena*, this species was transferred in 1988 to the genus *Enterobacter* after DNA-DNA hybridizations were studied for the three strains (46). Then, in 1989, Grimont and Ageron observed the synonymy between *E. cancerogenus* and *Enterobacter taylorae* (46). Currently, both denominations are still valid. Nevertheless, the name *E. cancerogenus* should be retained because it was established earlier. These strains are ODC and ADH positive and LDC and urease negative. They do not ferment D-sorbitol, sucrose, melibiose, dulcitol, and raffinose, and they are not gelatinolytic. Additional identification characteristics are presented in Table 1. Studied *E. cancerogenus* strains are generally susceptible to third-generation cephalosporins, moxalactam, imipenem, gentamicin, kanamycin, norfloxacin, ciprofloxacin, and colistin,

TABLE 1 Biochemical characteristics for the identification of species of the genus *Enterobacter*[a]

Organism	Yellow pigment	LDC	ADH	URE	ESC	Fermentation								
						INO	SOR	SAC	MEL	RAF	AMG	DUL	ADO	ARL
E. amnigenus biogroup 1	−	−	−	−	+	−	−	+	+	+	V	−	−	−
E. amnigenus biogroup 2	−	−	V	−	+	−	+	−	+	−	+	−	−	−
E. cancerogenus	−	−	+	−	+	−	−	−	−	−	−	−	−	−
E. asburiae	−	−	(−)	+/−	+	−	+	+	V	(+)	+	−	−	−
E. cloacae subsp. *cloacae*	−	−	+	−	−	(−)	+	+	+	(+)	(+)	(−)	(−)	(−)
E. cloacae subsp. *dissolvens*	−	−	+	+w	+	V	+	+	+	+	+	−	−	−
E. hormaechei subsp. *hormaechei*	−	−	(+)	(+)	−	−	−	+	−	−	+	+	−	−
E. kobei	−	−	+	+	−	+	+	+	+	+	+	V	−	−
E. ludwigii	−	(−)	+	−	−	+	+	+	+	+	+	−	−	
E. nimipressuralis	−	−	−	−	+	−	+	−	+	−	+	−	−	
E. aerogenes	−	+	−	−	+	+	+	+	+	+	+	−	+	
E. gergoviae	−	(+)	−	+w	+	−	−	+	+	+	−	−	−	+
E. mori	+	+	ND	ND	+	ND	+	ND	+	ND	ND	ND	ND	+
E. bugandensis	−	−	+	−	+w	+	+	ND	+	+	ND	+	−	ND

[a]Data are from references 4, 5, 12, 48, 55, 75, 82, 86, and 120. Abbreviations: LDC, lysine decarboxylase; ADH, arginine dihydrolase; URE, urease; ESC, esculin; INO, inositol; SOR, sorbitol; SAC, saccharose; MEL, melibiose; RAF, raffinose; AMG α-methyl-D-glucoside; ADO, adonitol; ARL, D-arabitol. +, positive reaction (>,90% of strains); (+), generally positive reaction; +w, weakly positive reaction; V, variable reaction; (−), generally negative reaction; −, negative reaction (<90% of strains); +/−, variable depending on the method used; ND, not determined.

and often they are susceptible to carbenicillin, ticarcillin, azlocillin, mezlocillin, and piperacillin. They are naturally resistant to aminopenicillins, some cephalosporins, and co-trimoxazole (47). They have an inducible chromosomal AmpC β-lactamase (45, 48–50).

Enterobacter cloacae Complex

The *Enterobacter cloacae* complex includes the following species: *Enterobacter asburiae*, *Enterobacter carcinogenus*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Enterobacter kobei*, *Enterobacter nimipressuralis*, and *Enterobacter mori*. All these species are genotypically very close, with more than 60% DNA-DNA homology. A phylogenetic study by Hofmann and Roggenkamp, based on sequences of four housekeeping genes, confirmed the genetic diversity of the *Enterobacter cloacae* complex, of which all affiliated species, although genetically related, form distinct clusters (3). *E. cloacae* and *E. hormaechei* are the most frequently isolated in human clinical specimens.

Enterobacter asburiae. *E. asburiae* was described in 1986 by Brenner et al. (5) from strains of the enteric group 17, of which atypical bacterial strains of the genus *Citrobacter* or of the genus *Enterobacter* had been collected since 1978. It is sometimes described as *Enterobacter muelleri*. These strains are sometimes immobile, often having a Voges-Proskauer (VP)-negative reaction. They are indole negative, they ferment D-sorbitol and sucrose, and they generally do not ferment melibiose. When they are VP positive, it is necessary to differentiate them from *E. cloacae* (*E. asburiae* does not have ADH and does not ferment L-rhamnose) and other VP-positive species (*E. asburiae* does not possess LDC, Tween 80 esterase, or DNase, characteristics possessed by *Serratia marcescens* and *Serratia liquefaciens*). Additional identifying characteristics are shown in Table 1. *E. asburiae* has occasionally been described as a bacterium with clinical significance, mainly in blood cultures. However, de Florio et al. observed its gradual increase in 2017 (51). The complete genome of a clinical *E. asburiae* isolate from a bone marrow transplant patient has been sequenced (52). The strains studied by Brenner et al. (5) were still susceptible to gentamicin and sulfadiazine and generally to carbenicillin, kanamycin, streptomycin, chloramphenicol, nalidixic acid, and colistin. They were all resistant to ampicillin, cefalotine, and tetracycline. Environmental strains isolated from watercourses in the United States have been shown to be resistant to imipenem by producing a plasmid-derived IMI-2 carbapenemase, thus confirming the hypothesis of an environmental reservoir of this type of resistance gene (53, 54).

Enterobacter cloacae. *E. cloacae* is the type species of the genus *Enterobacter*. It is now divided into two subspecies: *E. cloacae* subsp. *cloacae*, for which the esculin test

is negative, and *E. cloacae* subsp. *dissolvens*, with a positive esculin reaction (55). The strains are ODC and ADH positive and LDC negative (among the bacteria of the genus *Enterobacter*, only the species *E. cloacae*, *E. taylorae*, *E. kobei*, and possibly *E. amnigenus* have this profile of decarboxylases). They ferment D-sorbitol, sucrose, and melibiose. Additional characteristics are presented in Table 1. Some pathogenicity factors have been identified as hemolytic and leukotoxic membrane cell cytotoxins (56). With regard to epidemiological dissemination, several studies have confirmed that *E. cloacae* colonizations/infections correspond to dissemination of several clusters corresponding to known major multilocus sequences types and that no relationship exists with the geographical source. The various clones have a widespread dissemination and are continuously arising and expanding. Clinical isolates come from various sources and reservoirs, representing sampling from the diversity of the species in the population, and patients are potentially colonized in different ways before entering the hospital (57). *E. cloacae* is naturally resistant to ampicillin, amoxicillin-clavulanic acid, cephalothin, and cefoxitin by the low-level production of the Bush group 1 inducible natural cephalosporinase (class C). Ureidopenicillins and carboxypenicillins are active on at least half of the strains (12). In AmpC chromosomal cephalosporinase, derepression and constitutive production by mutation can lead to resistance to a large number of β-lactams, particularly third-generation cephalosporins, except for cefepime (12, 58, 59). This AmpC-type resistance accounts for 50% of β-lactam resistance in clinical strains and coexists frequently with that due to extended-spectrum β-lactamase (ESBL) expression.

In 1989, the first cases of nosocomial infections due to strains possessing an ESBL were identified (18). Since then, various ESBLs, such as TEM, SHV, and CTX-M, have been characterized in *E. cloacae*, including inhibitor-resistant TEMs (IRP) (60–63). *E. cloacae*, along with *Escherichia coli* and *K. pneumoniae*, is one of the most common *Enterobacteriaceae* resistant to third-generation cephalosporins. Nevertheless, in recent years, clinical isolates that are resistant through the production of carbapenemases have been identified (64–67). In particular, in Asia, strains harboring IMP, NDM, GIM, or KPC enzymes have been described (68–70). Lee et al. found an imipenem resistance rate of 0.4% in *E. cloacae* (71). For aminoglycosides, the percentages of resistant strains range from 0% to 51% for gentamicin and from 0% to 34% for amikacin, whereas ciprofloxacin is active in 64% to 100% of cases (12). A recent study showed that 77% of clinical strains in China are plasmid positive with determinants of aminoglycoside resistance (70). Regarding quinolones, *Enterobacter cloacae* is one of the enterobacteria, along with *Escherichia coli* and *Klebsiella pneumoniae*, in which resistance of plasmid origin due to the QnrA protein was initially observed (70, 72–74). These determinants of plasmid resistance to fluoroquinolones are found in more than 60% of the strains (70).

***Enterobacter hormaechei*.** *E. hormaechei* was designated by O'Hara et al. in 1989 to describe previously assembled strains in enteric group 75 (75). These are LDC- and gelatinase-negative strains that are generally ODC, ADH, and urease positive, and they ferment sucrose and L-rhamnose but not D-sorbitol or melibiose. These characteristics generally make it possible to differentiate this entity from phenotypically close species. Additional identifying characteristics are shown in Table 1. Hoffmann et al. have subdivided *E. hormaechei* into three subspecies based on the differential biochemical characteristics for D-adonitol, D-arabitol, D-sorbitol, D-melibiose, and dulcitol: *E. hormaechei* subsp. *oharae*, which ferments only melibiose; *E. hormaechei* subsp. *hormaechei*, which ferments only dulcitol; and *E. hormaechei* subsp. *steigerwaltii*, which ferments all of these compounds except for dulcitol (76). Two additional subspecies have now been characterized by whole-genome comparisons and average nucleotide identity from complete genome sequencing: *E. hormaechei* subsp. *xiangfangensis* and *E. hormaechei* subsp. *hoffmannii* (41).

Most strains described by O'Hara et al. have been isolated from samples of human origin. These isolates were sensitive or moderately sensitive to azlocillin, mezlocillin, piperacillin, cefotaxime, ceftriaxone, ceftazidime, moxalactam, imipenem, gentamicin, tobramycin, amikacin, chloramphenicol, co-trimoxazole, and trimethoprim (75). Rare strains harbored ESBLs and hyperproduced AmpC cephalosporinase, thereby having

resistance to third-generation cephalosporins (77). Strains producing carbapenemases have been recently identified (78). Davin-Regli et al. reported a nosocomial outbreak involving 21 isolates of *E. hormaechei* subsp. *oharae* which were resistant to fluoroquinolones, and their isolation was always preceded by treatment of patients with a fluoroquinolone (17).

Enterobacter kobei. The name *Enterobacter kobei* was proposed by Kosako et al. to group strains belonging to the NIH 21 group, which was previously included in *E. cloacae* (79). These strains differ from *E. cloacae* by a negative VP reaction. However, recently, a new VP-positive biotype causing urinary tract infection (UTI) has been characterized (77). The clinical significance of these strains is often uncertain. However, the species is now also of concern because of ESBL production (80).

Enterobacter ludwigii. The new species *Enterobacter ludwigii* has been described on the basis of genotypic and phenotypic characteristics of 16 strains with a clinical origin (81). It is genetically close to *E. hormaechei*, and a Biotype 100 Gallery API system was used to distinguish it from other *Enterobacter* spp. by its ability to use *myo*-inositol and methyl-D-glucopyranose. Whole-genome sequencing of the type strain was conducted (82). The strains are ADH and ODC positive and LDC and urease negative. All strains are naturally resistant to ampicillin, amoxicillin-clavulanic acid, and cefoxitin. Some strains of clinical origin hyperproduced AmpC cephalosporinase but were sensitive to cotrimoxazole, gentamicin, imipenem, and ciprofloxacin. Moreover, recently a nosocomial bloodstream infection outbreak in neonates caused by *Enterobacter ludwigii* coharboring CTX-M-8, SHV-12, and TEM-15 (83) and a clinical isolate responsible for a postoperative bone infection coharboring NDM-1 and OXA-48 carbapenemases in India (84) were described.

Enterobacter mori. *Enterobacter mori* was first described as a phytopathogenic bacterium (85). It was isolated from diseased mulberry roots. The species could be differentiated from closely related species by the presence of lysine decarboxylase activity and the ability to use D-arabitol. The type strain R18-2T (= CGMCC 1.10322T = LMG 25706T) was sequenced. The 16S rRNA gene sequence and MLSA indicated that *E. mori* is closed related to *E. asburiae* (*E. muelleri*) (4). Sixty-six genes potentially involved in the secretion system have been identified, explaining the phytopathogenic nature of this species (39). However, recently it has been identified as being responsible for human infection in Austria, and the isolates carried an IMI-2 carbapenemase (39).

Enterobacter nimipressuralis. *Enterobacter nimipressuralis* was first considered an *Erwinia* sp. before being reclassified as *Enterobacter* in 1986 by Brenner et al. (5, 86) on the basis of DNA-DNA hybridizations. Strains of *E. nimipressuralis* are not pigmented and are generally ADH positive and urease negative. They ferment D-sorbitol and melibiose but not sucrose. These characteristics most often make it possible to differentiate them from species that are phenotypically similar to the genus *Enterobacter*. Additional identifying characteristics are shown in Table 1. No isolate of human origin has been described except from a pseudobacteremia (87). However, Brenner et al. believed that this species could be involved in clinical practice because of the phenotypic proximity of these two species to *E. cloacae* (5); strains of clinical origin could be reported under that name. Hoffmann cluster X is *E. nimipressuralis*, and it has been suggested that it be reclassified as *Lelliottia nimipressuralis* by MLSA (3).

Enterobacter aerogenes

Enterobacter aerogenes phenotypically and genotypically resembles a mobile *Klebsiella pneumoniae* strain because of its peritrichous, ODC-positive, urease-negative, and indole-negative flagella (Table 1). Izard et al. proposed to reclassify *E. aerogenes* as *Klebsiella* under the name *Klebsiella mobilis* (88). While it is taxonomically justified (by DNA-DNA hybridization), this proposal has not been accepted by medical microbiologists, who maintain the name *E. aerogenes*. From genome sequencing performed on a resistant clinical isolate, Diene et al. (37) have recently proposed the shift of the *E. aerogenes* species to the genus *Klebsiella* as *K. aeromobilis*. The successive acquisition of additional genes from genetic mobile elements and other species, which are efficiently

integrated and translated, contributes to its notable phenotype (37). Interestingly, several genes involved in the bacterial mobility could have been borrowed from the *Serratia* genus, and the conjugative plasmid also could have been constructed from various transposons or genetic mobile elements (37).

E. aerogenes has been regularly involved in nosocomial infections since 1992, particularly in Western Europe (9, 13, 16, 89–92). In 2012, in France, *E. aerogenes* represented the fifth enterobacterium responsible for nosocomial infections and the seventh Gram-negative bacillus. Its prevalence has fallen sharply since 2000 (21). Although *Enterobacter cloacae* is now the *Enterobacter* sp. most frequently isolated in clinical settings and the species expressing the widest panel of new β-lactamases or carbapenemases, *E. aerogenes* more easily leads to septic shock in infected patients, is associated with higher mortality (39% of patients), and shows greater virulence (93, 94).

Strains of *E. aerogenes* have a broad ability to acquire antibiotic resistance mechanisms (95). They possess a low-level natural AmpC chromosomal cephalosporinase (Bush group 1) that induces resistance to first-generation cephalosporins. The hyperproduction of chromosomal AmpC in clinical strains leads, after induction by the third-generation cephalosporins or after mutation, to resistance to all β-lactams except cefepime, cefpirome, and carbapenems (96). The plasmid-borne AmpC cephalosporinase gene ($bla_{\text{CMY-10}}$) gives the same phenotypes (97). In 1993, the first cases of nosocomial infections caused by ESBL-producing strains were observed. In 1998, Pitout et al. isolated ESBL-producing strains resistant to gentamicin and co-trimoxazole (98). Various ESBLs were identified as being in the TEM, SHV, and CTX β-lactamases families, but TEM-24 remains associated with the preferential conjugative plasmid of this species (60, 99–104). In these producer strains, the sensitivity to carbapenems is generally preserved. In parallel, a number of imipenem-resistant clinical strains have been described (61, 93, 95, 99). In these isolates, the lack of antibiotic penetration is associated mainly with a modification of the porin expression: an alteration of the Omp35/Omp36 balance is detected and then followed by a total defect of the porins in the strains collected during the treatment (61, 93, 105–108). Interestingly, an original mechanism of impermeability has been reported, with the presence of a mutation in Omp36 that strongly alters the channel properties (106, 107). Finally, since 2008, carbapenemases of the IPM, NDM, or KPC type have been described as responsible for carbapenem resistance (95). Moreover, approximately 40% of multidrug-resistant (MDR) clinical strains have active efflux (108). Resistance to quinolones is due to modification of the target or due to plasmid-borne resistance (*qnrS* or *qepA*, encoding an efflux pump) transmitted by other species. Finally, total resistance is not an exceptional phenotype in *E. aerogenes*, since a strain resistant to all antibiotics, including colistin by mutation of *pmrA*, has been isolated and studied (37, 109).

Enterobacter gergoviae

Enterobacter gergoviae was described for the first time by Richard et al. in 1976 using a multidrug-resistant hospital strain isolated in Gergòvia near Clermont-Ferrand, France (110). Its classification was confirmed in 1980 by Brenner et al. after a DNA-DNA hybridization study (5, 111). Recently, a suggestion was made to include this species in the genus *Pluribacter* as *P. gergoviae*, based on the sequence analysis of four genes according to MLSA (35). The strains are generally LDC and ODC positive and gelatinase negative. They are urease positive and do not ferment inositol, D-sorbitol, and mucate, characteristics that differentiate them from *E. aerogenes*. Unlike other *Enterobacter* bacteria, *E. gergoviae* does not grow in potassium cyanide broth. Additional characteristics are presented in Table 1. *Enterobacter gergoviae* is rarely clinically isolated and has been exceptionally resistant to antibiotics (5, 35, 45, 112). Recently, however, ESBL type SHV and carbapenemase type (IMP or KPC) producers were described in this species (113). With regard to biocides, due to membrane modifications, esterase production, and the modulation of enzymes involved in oxidative detoxification, this species has a natural resistance to the parabens, triclosan, and methylisothiazolinone-chloromethylisothiazolinone (MIT-CMIT), which are preservatives used in this type of

product (114, 115). Such results explain the ability of this species to contaminate cosmetics from a source probably of unknown plant origin (116, 117).

Recent Species Descriptions

Enterobacter bugandensis, *E. timonensis*, *E. massiliensis*, *E. chengduensis*, *E. sichuanensis*, and *E. roggenkampii* were recently described based on a computational analysis of sequenced *Enterobacter* genomes or MLSA of housekeeping genes (20, 40, 41, 118, 119). *E. bugandensis* was responsible for a 3-month outbreak of septicemia in a neonatal ward in Tanzania and was also identified from the environment of the International Space Station and studied for its MDR phenotype (4, 118). On the basis of whole-genome sequencing, this species was found to be phylogenetically close to *E. hormaechei*. It is capsule-forming and motile, and its biochemical properties are presented in Table 1. All strains harbored a $bla_{CTX\text{-}M\text{-}15}$ gene and were resistant to quinolones, tetracycline, and sulfamides.

E. timonensis and *E. massiliensis* (characterized in the Timone Hospital laboratory, Marseille, France) were described on the basis of mass spectrometry (MS) and 16S rRNA DNA-DNA hybridization among strains isolated from the gut microflora of patients from Africa (40). These isolates were not associated with human infections. *Enterobacter chengduensis* and *E. sichuanensis* were isolated in China from a human blood sample and from urine, respectively, and considered to be new species due to particular phenotypic characteristics and by phylogenetic analysis using MLSA (4, 119). *E. roggenkampii* (rog.gen.kamp'i.i. N.L. gen. m. *Roggenkamp*) was named in honor of Andreas Roggenkamp, a German bacteriologist who helped with the understanding of the phylogenetic structure of the *E. cloacae* complex. This creates a novel clade of the *E. cloacae* complex, on the basis of cluster determination within the *E. cloacae* complex using *hsp60* genes as marker genes (41). *Enterobacter roggenkampii* sp. nov. is the type strain for Hoffmann cluster IV.

EPIDEMIOLOGY AND GLOBAL SPREAD

Environmental Sources

Members of the genus *Enterobacter* are environmental organisms and opportunistic pathogens of plants and humans. They are commonly found in water, sewage, soil, plants, or animal feces (120). *E. amnigenus* biogroups 1 and 2 have been isolated from drinking water, surface water, and wild soils. *E. asburiae* has been isolated from water, soils, plants, food, hospital environments, and health care staff equipment, such as probes, catheters, etc. (121). *E. cloacae* has been isolated from food, especially from samples of formula containing plants, raw vegetables, and roots, as well as from drinking water (45, 122). Dugleux et al. described an outbreak of *E. cloacae* septicemia in a hospital due to the contamination of parenteral nutrition preparations stored in a refrigerator (123). Similarly, outbreaks due to human albumin flasks (124), humidifiers and respiratory therapy equipment (125), and hydrotherapy water in a burn unit (126) have been described.

E. gergoviae has been isolated from the environment, from fruits and vegetables, from various sterility controls, and in batches of various types of cosmetic products (48, 110, 116, 117). The reservoir of *E. gergoviae* is unknown but could be associated with a plant biotope. Numerous *Enterobacter* species are endophytic bacteria and are present in plant rhizospheres.

Human Reservoirs and Hospital-Acquired Infections

E. amnigenus has been isolated from respiratory tract samples, wounds, and stool, as well as from a catheter and a series of blood cultures in a patient undergoing cardiac transplantation (43). *E. cancerogenus* has been found in specimens of cerebrospinal fluid, blood, osteomyelitis, bile, tracheal secretions, and urine, for which its clinical significance has been proven. It has also been isolated from skin specimens, without evidence that *E. cancerogenus* was responsible for the infection (49, 50, 127–129). In 1997, Abbott and Janda, reported 5 cases of *E. cancerogenus* infections as bacteremia

in patients with significant injuries or trauma (129). In one of them, *E. cancerogenus* was isolated for 2 months in the liquid drainage.

E. asburiae has been isolated from urine, respiratory samples, blood, stool, wounds, skin, and gallbladder (52). *E. cloacae* is present in the normal flora of the human gastrointestinal tract. This species is very often isolated in samples of clinical origin: urine, sputum, and blood culture (130, 131). Currently, the bacterium is found frequently during systematic sampling of neonates who have been colonized early (132). It is involved in 10% of postsurgical peritonitis cases, 5% of nosocomial sepsis and pneumonia cases, and 4% of urinary tract infections (3). Fata et al. reported a fatal case of myositis in a neutropenic patient (133). *E. cloacae* has been implicated in cases of endophthalmitis (134), brain abscess (135), meningitis (136), spondylodiscitis (137), and endocarditis (138).

E. hormaechei has been isolated from wounds, sputum, and blood cultures. In 1997, Wenger et al. (19) reported an outbreak in 1993 in an ICU involving 10 premature infants and including 5 cases of bacteremia.

Clinical strains of *E. kobei* have been isolated from various clinical samples: blood, sputum, urine, and especially intra-abdominal samples. Recently, a nosocomial bloodstream infection outbreak occurred in a neonatal ICU in a Venezuelan hospital and was caused by *Enterobacter ludwigii* coharboring three different ESBLs (84). *E. aerogenes* is quite frequently isolated in human samples (respiratory, urinary, blood, abscess, gastrointestinal, or cutaneous samples) or from materials such as ureteric stents. The species is isolated particularly from patients hospitalized in ICUs (15, 22, 23, 109, 139). The spread from patient to patient due to inadequate attention to infection control measures, especially handwashing, represents the main risk factor (16). Particular infections were described as endocarditis, endophthalmitis, and postneurosurgical meningitis (140).

Finally, *E. gergoviae* was isolated from respiratory samples, wounds, blood cultures, stools, and urine. Except for its involvement in a nosocomial epidemic of urinary tract infections in France in 1976 and an epidemic of bacteremia in newborns following the contamination of a parenteral glucose solution, sporadic cases have been described (141), such as pulmonary pneumopathy (142).

CLINICAL ASPECTS

Pathogenicity

Little is known about the pathogenicity and virulence factors of *Enterobacter* spp. due to the paucity of studies in this area. Like other enterobacteria, they possess a flagellum. In addition to facilitating motility, flagella possess several other functions: biofilm formation, protein export, and adhesion (143). *Enterobacter* spp. also possesses different endotoxins (12). Barnes et al. observed that *Enterobacter* species strains secreted *in vitro* enterotoxins, alpha-hemolysins, and cytotoxins similar to Shiga-like toxins II, "thiol-activated pore-forming cytotoxins" (144, 145). In Gram-negative bacteria, the type III secretion system (TTSS) is recognized as a pathogenicity factor. One study showed that 27% of *E. cloacae* isolates from clinical infections possessed this factor (10). The *E. cloacae* complex strains may also induce apoptosis of Hep2 cells (146). The acquisition of the plasmid pQC described by Paauw et al., containing virulence-encoding (*ter* and *sea*) and resistance-encoding ($bla_{CTX\text{-}M\text{-}9}$, *qnrA1*, *aadB*, *aadA2*, *sukK*, and *sat*) genes, contributes to the virulence and adaptation of *E. cloacae* clade 1 (10). Additionally, *E. hormaechei* has been reported to be more virulent than other species due to the presence of a high-pathogenicity island (HPI) that is frequently detected on its chromosome.

The ability of bacteria to assimilate iron through chelators is important for bacterial metabolism and for the establishment of infection. The siderophore-encoding genes are generally observed in HPIs, especially in *Yersinia* spp. Among these genes, the *irp2* gene has been identified in *Enterobacter* spp. (147). Finally, *E. cloacae* complex strains can harbor curli-encoding genes involving host cell adhesion and invasion. A recent study showed that 78% of the clinical strains studied ($n = 11$) had the *csgBA* operon

(which encodes curli). The authors observed a significant correlation between biofilm formation by these strains and gene expression of *csgA* (coding for the main subunit of curli, curlin) and *csgD* (coding for an activator of the operon) (148).

In the genus *Enterobacter*, some differences in pathogenicity could be noted between *E. aerogenes* and *E. cloacae* (149). Azevedo et al. reported the presence of virulence-encoding genes in *E. aerogenes* that have been identified in *Klebsiella pneumoniae* (149). For instance, the *fimH* and *mrkD* genes, encoding adhesins of type 1 and type 3 fimbriae, and *ycfM* are detected, and they play a key role in bacterial adhesion and in biofilm formation, which are important aspects of bacterial virulence (150). Regarding iron transport, *kfu*, *entB*, and *ybtS*, genes that are involved in the production of siderophores, are identified in *E. aerogenes* (151). In this regard, it is important to note that Kfu is often detected in hypervirulent *K. pneumoniae* strains, and the *allS* gene, which is involved in allantoin metabolism, is closely associated with *K. pneumoniae* isolates detected in liver abscesses. Finally, the virulence of TEM-24-producing *E. aerogenes* was evaluated in the *Caenorhabditis elegans* model (93). A significant reduction of *E. aerogenes* virulence was observed in resistant strains that have modifications of membrane permeability involved in drug resistance. This difference is noticeable even though this species exhibits a moderate virulence in this model, although the studied strains harbored the HPI virulence factor-encoding genes. The alteration of outer membrane (OM) permeability, e.g., a lack of porins that are a prominent entry pathway for nutrients, has an important impact on bacterial fitness. The antibiotic pressure promotes the emergence of resistant strains having porin deficiency and lipopolysaccharide (LPS) modifications that generate a nonphysiological membrane state (29). This causes an unfavorable fitness cost that consequently alters the infection/colonization capability (93).

Characteristics of Affected Patients

Enterobacter spp. are involved in nosocomial infections and especially in ICUs, where they affect immunocompromised patients, such as neonates, premature infants, patients with diabetes mellitus, burned or multiply traumatized patients, and patients with leukemia or who are undergoing immunosuppressive therapy. Invasive procedures, such as catheterization and intubation, which are frequently found in an ICU, represent a main source of infection (99, 152–155). The patients also harbored numerous comorbidities with a high Charlson score. Among them, diabetes mellitus and its main complications (chronic vascular and renal diseases) represent a risk factor for *Enterobacter* infection (152). Finally, *Enterobacter* spp. preferentially affect patients with a long median duration of hospitalization. This time increases the digestive carriage, which represents a high-risk factor for transmission (156, 157). A persistence of digestive carriage over at least a 5-year period was demonstrated (158).

Frequently, the acquisition of *Enterobacter* spp. concerned MDR strains. Nosocomial acquisition and the median number of antibiotics used represent risk factors for these bacteria (159). An ICU stay of $>$14 days, presence of a tracheostomy, prior central venous catheter use, prior receipt of mechanical ventilation, and previous exposure to broad-spectrum antibiotics or any antibiotic during the 30 days before the infection were also associated with acquisition of this MDR (160, 161). Due to the immune context of the patients and the high rate of multidrug resistance, the presence of *Enterobacter* spp. in the bloodstream represents a high risk of mortality (152, 154, 162).

Clinical Manifestations

Among *Enterobacter* spp., *E. aerogenes* and the *E. cloacae* complex have been described in various nosocomial outbreaks that correspond to more than 5% of the bacteremia acquired in the hospital, 5% of pneumonia, 4% of UTIs, and 10% of postsurgical peritonitis cases (12). *Enterobacter* spp. are involved in numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter related), and abdominal cavity/intestinal infections (24, 163). These species are especially described in ICUs, as previously mentioned, and

have also been involved in sepsis occurring in neonatology (164, 165). Moreover, *E. hormaechei* has also been identified in intravascular device-related infections, in surgical site infections (primarily postoperative in orthopedic trauma or related to devices), and, notably, after organ transplants (160, 166–171).

Within the *E. cloacae* complex, the most isolated species are *E. hormaechei* (clusters VIII and VI), with 40% to 48% of strains isolated, followed by *E. cloacae* cluster III, with 25% to 42% of strains being isolated (11, 34, 172). In 2012, Kremer and Hoffmann were interested in the types of infections caused by the different species of the group (172). They studied 196 strains which had been isolated from various samples: blood cultures; catheters; pleural fluids; and respiratory, urogenital, digestive, and cutaneous samples. In blood cultures, *E. hormaechei* subsp *oharae* (cluster VI) was significantly more prevalent, whereas *E. asburiae* was not represented at all. On the other hand, *E. asburiae* was more frequently isolated in respiratory samples than in samples from other sites. *E. hormaechei* subsp. *steigerwaltii* (cluster VIII) was overrepresented in skin injury swabs, particularly in burns. No clonality relationship was identified between the strains. Paauw et al. studied 156 strains and similarly showed that clade 1 was significantly more involved than clade 2 in infections, suggesting that this clade had greater pathogenicity (34). This clade is more common in the nosocomial environment, and its implication in infections could be the result of a better adaptation in this environment rather than a higher pathogenicity. This hypothesis is supported by the detection of the pQC plasmid in clade 1 but not in clade 2 species (10). Reports of several outbreaks of sepsis in neonatal ICUs in Brazil and the United States have been reported, with *E. hormaechei* being implicated (165). In 2016, Akbari et al. studied 50 *Enterobacter* strains isolated from UTIs (7). Twenty-five were part of cluster VI, 9 part of cluster III, and 6 part of cluster VIII. Clusters IV, X, XII, and XIII were absent (7). In 2009, the first study on the involvement of *E. cloacae* specifically in infected orthopedic implants was published (11). Fifteen strains (71%) belonged to *E. hormaechei* (5 of cluster VI and 10 of cluster VIII), 2 (9%) to *E. cloacae* cluster III, and 2 (9%) to *E. ludwigii* (cluster V). *E. cloacae* subsp. *cloacae* and *E. asburiae* were identified only once, and other species were not observed. The authors found a significant difference between the prevalence of cluster III in this type of infection and that of cluster III in the other samples. Cluster III was less commonly present in infected orthopedic implants compared to their overall distribution. In addition, in hip prosthesis samples, only *E. hormaechei* was isolated (9 out of 9). The authors hypothesized that different species would be implicated in different infections. Finally, *E. cloacae* was one of the most prevalent species isolated from diabetic foot infections using a culturomics approach (173). This result confirms its role in wounds and bone infections.

First-Line Antibiotics and Treatment

As infections due to *Enterobacter* are mainly nosocomial, most isolates present a broad resistance to third-generation cephalosporins, penicillins, and quinolones due to previous treatment of infected patients located in the same or next hospital ward. Some antibiotics remain effective for treatment: for instance, among the beta-lactams, the fourth-generation cephalosporins and carbapenems are the most attractive options, even if limiting carbapenem use should be encouraged, and the aminoglycosides have a good activity.

The use of third-generation cephalosporins and the monobactams (e.g., aztreonam) represents an important risk of *in vivo* derepression of AmpC β-lactamases, which can be due to a mutation in the repressor, during the treatment inducing high-level resistance to many β-lactam antibiotics. The interest in the concomitant use of aminoglycoside to prevent this type of resistance is mixed (9, 174). The use of fourth-generation cephalosporins (e.g., cefepime and cefpirome) seems to be more effective, mostly due to their activity against AmpC-hyperproducing *Enterobacter* strains (175). Compared to older cephalosporins, these molecules present (i) an efficient diffusion across the outer membrane porins, (ii) a significant stability against chromosomal β-lactamases, and (iii) an enhanced affinity for key penicillin-binding proteins located in

the *Enterobacter* periplasm (176, 177). Many publications have demonstrated interest in them (178, 179).

Carbapenems are very efficient against a wide variety of enterobacteria (180). AmpC-overproducing *Enterobacter* spp. typically remains susceptible to carbapenems. However, the use of carbapenems could induce a loss of porin production and an impermeability of the bacteria (see "Membrane-associated mechanisms" in the section Antimicrobial Resistance) (152). This resistance remains rare to date.

Recently, the use of the piperacillin-tazobactam combination has been observed to be a valuable treatment option for bloodstream infections due to *Enterobacter* spp. (178). Different new antibiotics have been tested against *Enterobacter* spp. The novel siderophore cephalosporin cefiderocol shows excellent results against these bacteria (181). Different combinations of cephalosporins and β-lactamase inhibitors (cefepime-zidebactam, cefepime-tazobactam, ceftolozane-tazobactam, ceftazidime-avibactam, etc.) also show high antibacterial efficacy against these pathogens (182–184). However, their use is not encouraged as a first approach because of the aim to limit the emergence of bacteria resistant to these new antibiotic solutions.

Finally, aminoglycosides, and particularly amikacin, remain active in more than 95% of *Enterobacter* spp. These rates have been stable over time. In *Enterobacter*, the aminoglycoside resistance is usually due to the presence of a plasmid coding for aminoglycoside-modifying enzymes (185).

ANTIMICROBIAL RESISTANCE

Development of Antimicrobial Resistance

Among *Enterobacter* spp., *E. cloacae* and *E. aerogenes* are mainly affected by development of antimicrobial resistance (14, 186). Regarding *E. aerogenes*, β-lactam uptake is closely associated with the presence of general porins, such as Omp35 and Omp36, which are homologous to the OmpC and OmpF porins that are the archetypes of the general nonspecific enterobacterial porins (187–190).

Several publications have described a modification of the porin pattern present in antibiotic-resistant isolates: resistant *E. aerogenes* can exhibit a shift in the type of porin expressed (Omp35 to Omp36), a reduction in the production level, or the synthesis of a porin exhibiting mutations in the porin structure that alter channel functions (for reviews, see references 29 and 188). These interplays of membrane impermeability and enzymatic barriers were first mentioned in H. Nikaido 's model (191, 192).

Consequently, the clinical isolates collected in the patient during antibiotherapy show a serious loss in susceptibility to cephalosporins and carbapenems (193, 194). This alteration of porin profiles is also often reported with a concomitant synthesis of degradative enzymes such as β-lactamases, cephalosporinases, or carbapenemases, which generate a worrying level of β-lactam resistance (193, 195–197).

Moreover, the dissemination of resistance genes via genetic mobile elements is an important aspect of the antibiotic resistance in Gram-negative bacteria in the ESKAPE group (14, 198).

Molecular Mechanisms of Resistance

A summary of the varieties of acquired resistance described in *E. cloacae* complex bacteria is given in Table 2.

Enzymatic barriers and epidemiology. In most *Enterobacter* spp., the production of β-lactamases is the prominent mechanism responsible for β-lactam resistance, and *E. aerogenes* and *E. cloacae* have a broad ability to modulate these mechanisms of resistance. Importantly, these bacteria are able to produce a low level of a chromosomal AmpC β-lactamase-type cephalosporinase that generates a resistance to first-generation cephalosporins (24, 196). The chromosomally acquired resistance promotes the overproduction of this AmpC cephalosporinase, for instance, during incubation with a subinhibitory concentration of carbapenem (199). Following the inactivation of AmpR or the acquisition of a plasmid-borne *ampC*, an overproduction of AmpC β-lactamase contributes to the resistance toward the third-generation cephalosporins (24, 200–202).

TABLE 2 Acquired resistances described in *Enterobacter cloacae* complex bacteria

Antibiotic group	Mechanism of resistance	Gene(s)	Species	Reference(s)
β-Lactams	Enzymatic β-lactamases			
	Class A	bla_{TEM}, bla_{SHV}, bla_{CTX-M}	*E. cloacae*	63, 315
			E. hormaechei	270
		bla_{VEB}, $bla_{GES/IBC}$, bla_{KPC}, bla_{FRI}	*E. cloacae*	210, 316
			E. hormaechei	317
		bla_{NMCA}, bla_{IMI}	*E. cloacae*	318
			E. asburiae	54
	Class B	bla_{VIM}, bla_{GIM}	*E. cloacae*	69, 319, 320
		bla_{NDM}	*E. ludwigii*	84
		bla_{IMP}, bla_{NDM}	*E. cloacae*, *E. hormaechei*	209, 321
	Class C	*ampC*	*E. cloacae*	288, 295
			E. asburiae	3
			E. hormaechei	213
			E. kobei, *E. ludwigii*, *E. nimipressuralis*	163
	Class D	bla_{OXA-48}	*E. cloacae*	320
	Impermeability/efflux	*acrAB-tolC*, *ompC*, *ompF*, *ompX*	*E. cloacae*	25, 31, 240, 241, 263, 322
Fluoroquinolones	Target mutation	*gyrA*, *gyrB*, *parC*, *parE*	*E. cloacae*	291
	Enzymatic (acetyltransferase)	*aac(6′)-Ib-cr*	*E. cloacae*	148
	Protective mechanism target	*qnr* (A, B, S, C, D)	*E. cloacae*, *E. hormaechei*	323
	Efflux	*qepA*, *acrAB-tolC*, *oqxAB*, *sugE*, *emmdR*	*E. cloacae*	258, 259, 263
Aminoglycosides	Enzymatic (acetyl, phospho, nucleotidyltransferase)	*aac*, *aph*, *ant*	*E. cloacae*	323
	Methylase	*armA*, *rmtB*	*E. cloacae*	324
Cyclines	Efflux	*acrAB-tolC*	*E. cloacae*, *E. hormaechei*	325

E. aerogenes is also able to integrate a large plasmid (130 kb) that contains the *ampC* gene of chromosomal origin (bla_{CMY-10}). In the absence of antibiotic pressure, this genetic transmission can contribute to a systematic spreading of the resistance mechanism (203). This AmpC-related resistance, described in 50% of clinical isolates, is frequently associated with the expression of ESBLs (24).

The first hospital-acquired infections caused by these strains that exhibit resistance to common β-lactams due to the expression of ESBLs were reported in 1993 (98). The TEM-24 enzyme was associated with *E. aerogenes* clonal dissemination in hospitals in France (8, 23, 102, 103). Other TEM types or CTX-M types (e.g., CTX-M-2) are also often reported, but TEM-24 remains associated with preferential conjugative *Enterobacter* plasmids (10, 24, 73, 99, 100). These enzymes contribute to a global resistance toward all β-lactams except carbapenems (62). In *Enterobacteriaceae*, *E. cloacae* is now identified as the third most common bacterium resistant to third-generation cephalosporins, after enteric *E. coli* and *K. pneumoniae* (204). Different enzymes (ESBLs) belonging to the TEM, SHV, and CTX-M classes have been characterized in *E. cloacae*, and these also include resistant TEM inhibitors or inhibitor-resistant TEM (IRT) enzymes. Notably, some variants that exhibit CTX-M production have been identified, and others, such as TEM or SHV, have been described from epidemic episodes (24). A transfer of a genomic resistance island is also possible in *Enterobacter* spp. For instance, a variant of AGI1 that belongs to the *Salmonella* genomic island/*Proteus* genomic island/*Acinetobacter* genomic island family has been recently described in *E. cloacae* (205). This isolate was resistant to all the antibiotics tested except imipenem and amikacin.

Imipenem is the most effective antibiotic for the treatment of *E. cloacae* infections (24). Carbapenemases that belong to the NDM and VIM types have been identified in *E. aerogenes* and *E. cloacae*. KPC or class D β-lactamases possessing carbapenemase properties, such as the OXA-48 type, have been identified in Europe, Asia, and America (24, 206, 207). In 2010, the CDC reported the first carriage of NDM-1-producing *E. cloacae* in patients previously treated in India (208, 209). Recently, metallo-β-lactamases

that comprise IMP-type enzymes and NDM-, GIM-, VIM-, and serine-carbapenemase-type KPC and FRI have been characterized (24, 210, 211). OXA-48-type serine carbapenemase seems to be the most prevalent (206, 212). In *E. cloacae*, an increase in the rate of imipenem resistance has been observed. Moreover, an epidemic survey of *E. cloacae* blood infection reported the presence of metallo-β-lactamases (24). *E. cloacae* is the third *Enterobacteriaceae* in the production of carbapenemase, and several strains have been described to simultaneously express two carbapenemases (213). A report mentioned that the KPC enzyme was the most frequently identified in the SMART global surveillance program from 2008 to 2014 (214). This KPC prevalence was also reported in another study, indicating a limited emergence of NDM-1 (215). In contrast, a longitudinal study (2013 to 2017) performed in China indicated that an NDM producer is predominant in *E. cloacae* (216). Moreover, the carbapenem resistance in the *E. cloacae* complex had noticeably increased in a recent study of carbapenem susceptibility performed by the U.S. Veterans Health Administration from 2006 to 2015 (217). A recent publication based on genomic epidemiology of carbapenemase-producing *Enterobacter* spp., comprising predominantly *E. xiangfangensis* and *E. hormaechei* isolates, reports that the most common enzyme identified is VIM, followed by NDM, KPC, OXA-48, and IMP (218). Finally, an association of carbapenemase production with a loss of porin expression has been demonstrated in strains resistant to combinations of β-lactamase inhibitors (relactam) plus carbapenems (219).

Regarding aminoglycoside resistance, aminoglycoside-modifying enzymes are distributed among acetyltransferases (aminoglycoside-*N*-acetyltransferases [AACs]), phosphotransferases (aminoglycoside-*O*-phosphotransferases [APHs]), adenylyltransferases (aminoglycoside adenylyltransferases [AADs] or aminoglycoside-*O*-nucleotidyltransferases [ANTs]), and 16S rRNA methyltransferases, such as ArmA and RmtB (70). They are often plasmid encoded or associated with transposable elements, which facilitate the acquisition of resistance phenotypes (163).

The aminoglycoside-modifying genes, e.g., *aac(3)-IIa*, *aac(6′)-Ib*, and *ant(2″)-Ia*, are involved in aminoglycoside resistance affecting, at different levels, tobramycin, gentamicin, and amikacin. The clinical strains frequently contain more than one enzyme (70, 185, 220). The enzymatic resistance to fluoroquinolones has been characterized as a two-point mutation allele of *aac(6′)-Ib* [named *aac(6′)-Ib-cr*], the aminoglycoside resistance enzymatic determinant, which became able to acetylate ciprofloxacin and norfloxacin (70, 221). The association with the $bla_{\text{OXA-1}}$ gene in various genetic mobile elements contributes to a rapid spreading of this new mechanism (24).

Membrane-associated mechanisms. Numerous imipenem-resistant clinical strains have been described, and these present a severe alteration of porin expression associated or not with the overexpression of efflux pumps that occurs during antibiotic therapy (24, 29).

Membrane-associated mechanisms of resistance, including porin defects and increased levels of efflux pumps, are now recognized to strongly participate in the MDR phenotype by controlling the internal concentration of antibiotics (29). These "concentration barriers" can also induce the emergence/induction of other mechanisms, such as target mutations (e.g., mutated gyrase) or the expression of detoxifying enzymes, including β-lactamases (193). Interestingly, the alteration of LPS also has been described in many resistant isolates (222, 223).

(i) Omps, porins, and OM permeability. OmpA was first reported and characterized in 1983, and the Tsx channel involved in nucleoside uptake was reported in 1997 in *E. aerogenes* (224, 225). Today, three general (nonspecific) porins, Omp35, Omp36, and Omp37, have been identified in *Enterobacter* spp., and two additional specific porins, LamB and PhoE, have been identified and exhibit some similarities with *E. coli* OM proteins that have been largely studied (for reviews see references 187–189, and 226). Importantly, due to their specific trimeric organization in the membrane, these OM proteins need important posttranslational steps that (i) perform the maturation of precursor forms, (ii) correctly insert the protein into the OM, and (iii) manage the rate and dynamics of the final trimeric assembly of newly synthesized proteins (227–229).

TABLE 3 Some examples of the membrane proteins identified in *Enterobacter* spp.

Bacterium	Protein	Function	Characteristics	Reference(s)
E. cloacae	OmpE35	General porin	Trimer	230, 233
	OmpE36	General porin	Trimer	230, 233
	OmpE37	Quiescent porin	Trimer	
	OmpX		Monomer	240
	OmpA	OM architecture	ND[a]	
	LamB			326
	PhoE			242
	EmmdR	IM transporter		258, 259
	SugE	IM transporter		258, 259
	TolC	OM channel	Trimer	254
	AcrA	Adapter	ND	254
	AcrB	IM transporter	Trimer	254
E. aerogenes	Omp35	General porin	Trimer	230, 243
	Omp36	General porin	Trimer	230, 243
	Omp37	Quiescent porin	Trimer	C. Bornet, unpublished data
	OmpX	Small channel	ND	239
	OmpA	OM architecture	Monomer	199, 224
	LamB	Maltoporin	Trimer	238
	PhoE	Phosphoporin	ND	ND
	Tsx	Nucleoside uptake	ND	225
	TolC	OM channel	Trimer	253
	AcrA	Adapter	ND	253
	AcrB	IM transporter	Trimer	253
	EefC	OM channel	Trimer	327, 328
	EefA	Adapter	ND	327, 328
	EefB	IM transporter	Trimer	327, 328

[a]ND, not determined.

Like the archetypes OmpC and OmpF, Omp35 and Omp36 are assembled in stable trimers, and each subunit contains a hydrophilic channel presenting a typical β-barrel structure organizing an internal eyelet that constricts the lumen and creates a strong transverse electric field guiding the diffusion of charged molecules. Recently, the three-dimensional (3D) structures of *E. aerogenes* and *E. cloacae* porins have been solved, and the trimeric structures have been published. Their structures exhibit a high sequence identity, and their channel properties, e.g., conductance and selectivity, which are determined by their planar lipid bilayers, are very similar; for instance, the transversal electric field located in the lumen of the channel is well conserved in the OmpF group, which is more permeable to anionic compounds (230). Regarding the OmpC group, which includes Omp36, OmpE36, and OmpK36 for *E. aerogenes*, *E. cloacae*, and *K. pneumoniae*, a smaller lumen of the pore, a lower conductance (approximately 3 nS), and a higher cation selectivity (with a P_{K^+}/P_{Cl^-} ratio of approximately 2.1 to 2.2) are obtained compared to those obtained from the OmpF orthologs (230). Importantly, the channel is able to promote antibiotic travel across the OM and ensure accumulation inside the periplasmic space, as demonstrated with purified Omp36 or by using intact cells and labeled antibiotics (231, 232). Moreover, immunological and functional comparisons of *E. aerogenes* and *E. coli* porins have reported some conserved and variable features in the antigenic profile and in the reception/translocation functions for bacteriocins (233). This illustrates an adaptive evolution of specific exposed domains when the pore activity is preserved (230) (Table 3).

Similarly, to the case for *E. coli* porin expression (28, 234, 235), the regulation of these OM general channels is sophisticated in *Enterobacter* spp., and several models have been proposed to integrate not only the Mar and Ram regulation cascades but also the two-component system (TCS) regulator pathways that are directly involved in the sensing, transmission, and control of porin transcription, translation, and assembly (24, 29, 193). These different means of regulation are involved in the *Enterobacter* response and adaptation to the presence of antibiotics; they represent the first barrier

and the main lever for controlling the penetration flux and accumulation level of antimicrobials [see "(iii) Efflux pumps and antibiotic activity" below].

(ii) Porins and antibiotic activity. Interestingly, a pioneer study has determined that the porin-deficient phenotype is present in approximately 6 to 7% of the β-lactam-resistant *E. aerogenes* isolates collected during a 1-year period (104, 236). During antibiotic treatment of patients, a sequential alteration in the balance between Omp35 and Omp36 has been reported: from the expression of Omp36 and 35 in the carbapenem-susceptible isolate, to the Omp36-producing strain lacking Omp35 and exhibiting intermediate susceptibility, and finally to a carbapenem-resistant isolate having no porins (93, 237). Furthermore, LamB porin can be expressed in place of Omp35 and Omp36, generating a low susceptibility to β-lactams (238). In some isolates, OmpX, a small OM protein, is involved in the downregulation of porin expression that is associated with a decrease of antibiotic susceptibility (239–241). An *E. cloacae* PhoE porin has been purified and characterized; however, no information has been obtained regarding its role in clinical strains (242). Resistant isolates collected from patients who received antibiotherapy were extensively studied with regard to their levels of porin expression and pore activity (106, 107, 199, 238, 242). The loss of porins has been reported in many studies carried out on *E. cloacae* and *E. aerogenes* clinical isolates, and due to space limitations, only a limited part of the published studies are indicated here (10, 200, 219, 243–248).

Importantly, a key mutation has been identified in a resistant isolate: this mutation, causing a Gly-to-Asp change located in the eyelet region of the Omp36 lumen, induced a strong modification inside the channel conformation, causing alteration of both conductance and selectivity. Consequently, the mutated porin promotes a noticeable resistance to β-lactams but preserves a limited nutrient permeation through the porin (106, 107). This selected "porin strategy" maintains a minimal cost fitness for the bacterial cell associated with a solid decrease of antibiotic diffusion contributing to the resistance (249). Interestingly, as previously mentioned, the porin loss has an impact on the pathogenicity of *E. aerogenes* isolates, which become less virulent in a *Caenorhabditis elegans* model (93). This adverse effect of resistance mechanisms on *K. pneumoniae* virulence has been extensively analyzed in the case of OmpK36 and OmpK35 in a recent review (250, 251). Moreover, similar observations have been reported for *E. coli* (252), and we may hypothesize that the porin expression is necessary for some important steps involved in virulence or contributes to the envelope stability during colonization or virulence.

In addition to general porins, TolC, the outer membrane channel involved in the efflux activity and secretion (RND), has been described and characterized in *E. aerogenes* and *E. cloacae* (251–253). In addition, *E. aerogenes* TolC and EefC have been documented and present different channel properties determined by using electrophysiology assays (254).

(iii) Efflux pumps and antibiotic activity. Multidrug efflux pumps present on the *Enterobacter* genomes belong to the ABC, MF, SMR, MATE, PACE, and RND superfamilies described previously (255, 256); for a classification of membrane transporters, see reference 257 and the Paulsen site (http://www.membranetransport.org/transportDB2/index.html) (Table 3). Inner membrane (IM) transporters correspond to a single membrane protein located in the inner bacterial membrane, and they function as monomers or as dimers (255). These IM transporters pump out the drugs from the cytoplasm (or the inner leaflet of the IM) to the periplasmic space, such as reported for EmmdR or SugE in *E. cloacae* (258, 259). Interestingly, these IM transporters belonging to the SMR or MATE families can cooperate with the RND family in order to efficiently expel antibacterial compounds outside the bacterial cell (260). In the bacterial envelope, an RND complex, the tripartite efflux system that comprises an inner membrane transporter, a periplasmic adapter protein, and an outer membrane channel, recognizes and translocates the drugs across the OM to the external medium (255, 256, 261). These RND efflux pumps contribute to the removal of a large number of chemically diverse

compounds, such as antibiotics, detergents, biocides, preservatives, etc., that are present in the bacterial volume and can be deleterious to the bacteria (255, 262).

With regard to the AcrAB-TolC pump, the complex has been identified and described in *E. cloacae* and *E. aerogenes* (253, 254, 263). Purification and biochemical characterization have been performed for TolC and EefC of *E. aerogenes*, and their channel properties have been documented (264, 265). Regarding the involvement of this efflux pump in the resistance of clinical strains, various publications have reported the expression of AcrAB-TolC and its contribution in the reduced susceptibility of the isolates produced (199, 237, 238, 253, 266). Finally, the protein AcrZ, which has been described in the *E. coli* AcrZ-AcrAB-TolC efflux pump, has also been characterized in the *Enterobacter/Klebsiella* genome (267, 268).

Recently, the OqxAB operon has been identified in *E. cloacae* and *E. aerogenes* strains, and this efflux pump contributes to a decreased susceptibility to quinolones in *Enterobacteriaceae* (269). In addition, selective efflux pumps also have been identified and described to play a role in heavy-metal resistance/tolerance in *E. hormaechei* and *E. asburiae* isolates (270).

An important point is the relevance and the prevalence of AcrAB-TolC in clinical isolates. In a study published in 2008, the evaluation of efflux activity, measured by using an efflux pump inhibitor (PAβN) in clinical isolates collected within an 8-year period (1995 to 2003), indicated a noticeable increase of efflux expression during this interval (108). Moreover, this study clearly pinpoints the importance of evaluating the prevalence of membrane barriers, e.g., impermeability due to porin loss or/and efflux expression, in clinical strains submitted to antibiotherapy treatment, as recently mentioned (265). This aspect is important when taking into account the role of AcrAB-TolC in the susceptibility of *E. aerogenes* to macrolides (271).

A main concern is the correlation that exists between efflux activity and intracellular accumulation of antibiotics (209). A series of publications focused on fluoroquinolone accumulation in *E. aerogenes* and *E. coli* strains expressing or not the AcrAB-TolC pump has clearly illustrated the impact of the efflux expression on the accumulation rate inside the bacterial cell (272–275). The expression of the AcrAB-TolC complex is able to maintain the internal concentration of antibiotics under the threshold required for triggering the bacterial killing (263, 276). Moreover, by using a microspectrofluorimetry method, the authors found that the fluorescence drug signal varied among the individual bacteria in a uniformly treated population (272, 273). These important data illustrate the heterogeneity of the intrabacterial accumulation of an antibiotic during early incubation times. This heterogeneity may reflect different levels of resistant phenotypes coexisting in the isogenic population due to different growth phase or division steps (272, 277). This may pave the way for identifying the bacterial adaptation and persister formation inside a bacterial population subjected to antibiotic stress (272, 273).

In addition, these RND pumps are involved in the bacterial pathogenicity and in the acquisition of additional mechanisms of resistance in *Enterobacteriaceae* (195, 262, 265). Using a mouse model for measuring the competitiveness and virulence of AcrAB-TolC parental or deletion *E. cloacae* strains, G. Bou's group has clearly demonstrated the involvement of this pump in the bacterial physiology (278).

(iv) LPS modification and polymyxin susceptibility. Various alterations of the OM structure are also associated with the LPS modifications in *Enterobacter* clinical isolates that induce some changes in polymyxin susceptibility (109, 279, 280). In some cases, the plasmid-mediated colistin resistance *mcr-1* has been identified in resistant *E. aerogenes* and *E. cloacae* strains (280–283). A study reports that the overall prevalence of colistin resistance corresponds to 0.67% of the total enterobacterial isolates collected during a 4-year period. The colistin resistance was higher in *E. cloacae* (4.2%) than in *E. coli* and *K. pneumoniae* (0.5% and 0.4%, respectively). Although the authors reported that this resistance was not associated with the *mcr* genes, unfortunately, the molecular and genetic characterization of this resistance is lacking (284). In a recent study, Guérin et al. analyzed a collection of 124 strains of the *E. cloacae* complex and concluded that the

PhoP/PhoQ TCS would play a role in colistin resistance regulation (285). This observation is similar to work performed on *E. aerogenes* clinical isolates collected from patients receiving imipenem (237). In a polymyxin-resistant strain, the authors identified mutations located on *phoQ* and *pmrB*, which are part of the well-described TCS controlling the LPS-modifying enzymes (237). The genome of the colistin-resistant strain identified in 2005 has been sequenced and analyzed, and a mutation in *pmrA* has been identified as the cause of the alteration of LPS biosynthesis that had been previously observed (37, 109). This type of chromosomal mutation that efficiently alters the OM structure and generates a noticeable decrease of polymyxin activity may be involved in the emergence of resistant strains devoid of *mcr-1* and *mcr-2* (247). Moreover, the dynamic of colistin resistance in *E. cloacae* during selective decontamination of the digestive tract in intensive care units has been recently reported, suggesting a possible clonal transmission (286).

Mutations in antibiotic targets. Regarding β-lactam resistance, the target mutation occurs rarely in *Enterobacter* spp. However, the diverse β-lactamases reported today are the result of a series of mutations that have successively appeared in the original β-lactamase genes (287). Moreover, mutations affecting the *ampR* gene have been described in strains where AmpC cephalosporinase is derepressed (200, 288, 289). In MDR *Enterobacter* isolates, multiple point sequence alterations can be found in *ampC* but are not in regions corresponding to the serine active site or the β-lactam-binding site and have no correlation with the resistance phenotype (246). However, amino acid deletions in the Ω loop of *E. cloacae* AmpC involved in enzyme competitiveness and point mutations suspected to affect the enzymatic activity were found in strains selected with ceftaroline-avibactam (290).

Regarding the mutations that contribute to antibiotic resistance, the best characterized are those that affect the quinolone target and, as found more recently, those involved in polymyxin resistance (see the previous section). In *Enterobacter* spp., mutations located in the quinolone resistance-determining regions (QRDRs) of targeted enzymes, e.g., gyrase or topoisomerase, have been described to confer high-level resistance (24, 237, 291–293). This is the common resistance mechanism identified in clinical isolates with recent descriptions of plasmid-mediated quinolone resistance (70, 72, 73, 294, 295). Recent studies in South Africa reported that *qnr* genes were commonly detected in resistant *Enterobacter* isolates collected in a hospital (296, 297). Interestingly, recently the *qnrE1* gene was reported as probably originating from the *Enterobacter* chromosome (298). This "target-protective mechanism" confers low-level resistance to first-generation quinolones when present alone (72–74). Importantly, these mechanisms exhibit a noticeable spread and have been reported in approximately 60% of clinical isolates due to the presence of various genes coding for ESBLs or AmpC-type β-lactamases on the same plasmid (70, 289, 295).

Lastly, MDR has recently been described in *Enterobacter* isolates (*E. cloacae* and *E. aerogenes*) and in MDR-associated porin alteration, target mutation β-lactamase production, and efflux overexpression that are accumulated during antibiotic treatment (24, 237). Some mechanisms are intertwined and controlled by regulators in a complex genetic cascade.

MDR and genetic regulation. Recently, several chemical inducers that are able to modulate the expression of *Enterobacter* membrane transporters, including porins and/or efflux pumps, have been described, e.g., salicylate, chloramphenicol, etc. (24, 193, 239, 299). Interestingly, the regulation of porin expression is a fast event, occurring shortly after the addition of chemicals in the culture medium or with the addition of subinhibitory concentrations of antibiotics. During incubation with low imipenem concentrations, an increase in the efflux pump expression that is mediated by the overproduction of MarA has been observed (104, 243, 299).

A schematic representation of the regulation pathways that control the expression of porins and efflux pumps and their interconnection in *Enterobacter* spp. is shown in Fig. 1.

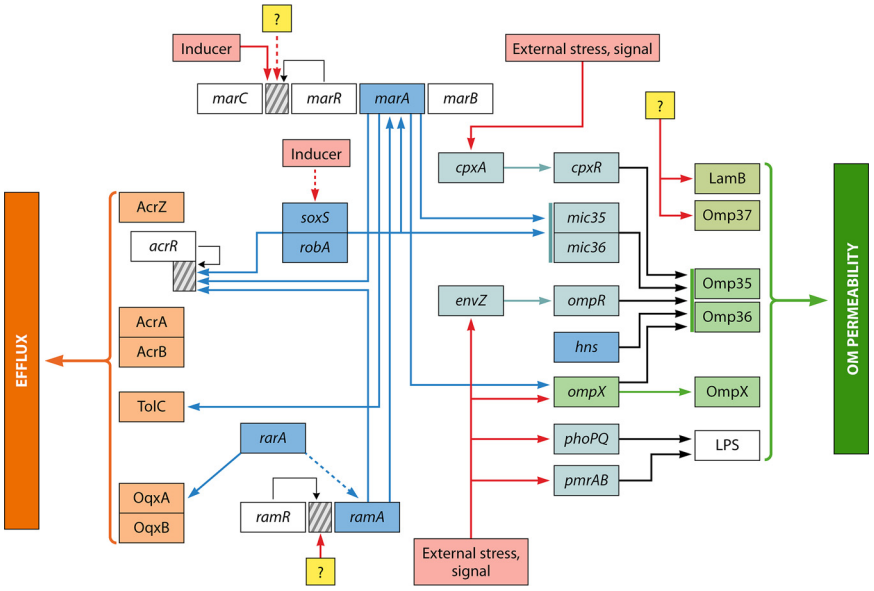

FIG 1 Schematic representation of the regulation pathways that control the expression of porins and efflux pumps and their interconnection in *Enterobacter* spp. The multiple regulation cascades that can modulate the outer membrane permeability (porins and LPS) and the expression of major efflux pumps (AcrAB and OqxAB) are summarized. Blue arrows represent transcriptional activation/repression of different genes, such as *acrR* and *ompX*, by the global regulators (e.g., Mar, Ram, and Sox). Red arrows symbolize the external stress signals that can activate/repress some gene expression. Black arrows indicate the negative regulation of gene expression (thin line directly on promoter/operator region). Yellow squares illustrate some unknown regulation that can modulate gene expression on promoter/operator regions (represented by dashed boxes) of key loci. Dashed lines represent hypothesized regulation.

(i) MarA, RamA, SoxS, and RobA. Regarding global regulators involved in the control of antibiotic resistance, importantly, the RamA regulator has been characterized in *Enterobacter*, *Salmonella*, and *Klebsiella*, but it has not been detected in *Escherichia*, in contrast to the case for the Mar regulon (24, 26).

RamA has been detected in *E. aerogenes* and *E. cloacae* and has generated a noticeable resistance to various antibiotics (chloramphenicol, tetracycline, tigecycline, fluoroquinolones, trimethoprim, etc.), in conjunction with a decreased expression of Omp35 and an active efflux in *E. aerogenes* (24, 271). RamA seems to be a "superregulator" of the membrane permeability, acting directly or via MarA and controlling the influx and the efflux of antibacterial agents in *Enterobacter* (24, 30). In addition, *rarA* may also contribute to the combined regulation of the RamA-MarA cascade during the emergence of antibiotic resistance (300, 301). RarA, which belongs to the AraC-type transcriptional regulators, is overproduced when the negative regulator OqxR is inactivated (25, 302). SoxS and Rob can also play a role by sharing some information detected via other signaling systems in *Enterobacter* (25, 278). Regarding environmental stress, H-NS (histone-like structuring nucleoid protein) modulates the level and balance (e.g., the Omp35/Omp36 ratio) of porins in the outer membrane in response to osmotic stress (24, 25).

(ii) Other regulators. With these global regulators, several other partners play a key role in monitoring the expression of porins: OmpX, a small OM protein, and different small noncoding RNAs (sRNAs), such as Mic35 and Mic36 (239–241). In addition, several TCSs, such as EnvZ-OmpR, and CpxA-CpxR, can regulate the expression level of porins. In parallel, other TCSs, PmrA-PmrB and PhoQ-PhoR, involved in the synthesis of LPS, which is involved in the last step of porin assembly in the OM, may also modulate the porin level in the OM. Importantly, some mutations have been identified in TCSs, such as PmrAB or CpxAB, indicating that under antibiotic treatment, clinical strains are able to select mutations that can modify the membrane permeability in order to acquire low susceptibility against antibiotics that are used (237).

Several local regulators, repressors such as *acrR* or *rarR,* play a role by controlling the expression of efflux pumps; a more detailed description of the regulators involved in the control of drug membrane transporters in *Enterobacter* and *Klebsiella* has recently been published (25), and an illustration of this complex network is presented in Fig. 1 This illustration has been constructed by using the publications on this subject and descriptions of the genes and proteins in the data bank.

(iii) Inducers and chemical effectors. Some inducers are described as binding directly to the repressor MarR, such as salicylate and tetracycline, thereby impairing the repressor action (25). The transcriptional activators respond to a variety of chemically unrelated compounds, including antibiotics, biocides, carbonyl cyanide *m*-chlorophenylhydrazone, cyclohexane, salicylate, acetylsalicylate (aspirin), acetaminophen, sodium benzoate, paraquat, and phenolic rings (24). However, the exact mode of action of this induction is not yet elucidated, and further investigations are necessary to understand the targeted step in the regulation cascade (Fig. 1).

DIAGNOSIS OF SPECIES

The routine identification of *Enterobacter* spp. has classically been performed by an evaluation of their phenotypic characteristics by using commercialized systems: the Api 20E gallery or Vitek 2 (bioMérieux). Although these systems are adapted to differentiate *E. aerogenes* and *E. cloacae*, they are not able to differentiate the species in the *E. cloacae* complex except for *E. cloacae* and *E. asburiae*.

Mass spectrometry, which is increasingly used today, can identify the *E. cloacae* complex but fails to differentiate the species within the group. The two most commonly used devices have failures with this identification. *E. nimipressuralis* is identified correctly, while, on the other hand, *E. hormaechei*, *E. cloacae*, *E. asburiae*, *E. kobei*, and *E. ludwigii* are not discriminated (51, 303).

Molecular biology techniques are needed to identify the species precisely. Sequencing of 16S rRNA is widely used to identify *Enterobacter* spp., but this technology cannot differentiate two closely related species; thus, it is not applicable to the case of the *E. cloacae* complex (40). The technique of microarray comparative genomic hybridization (microarray-CGH) is also a powerful identification method, but this method is time-consuming, expensive, and therefore difficult to implement routinely. It showed the existence of two distinct, genetically different clades within the complex. Possibly, this method could be coupled with MLSA. The first clade is then divided into two clusters, with the second being more heterogeneous and containing five clusters. Most strains associated with infections belong to the first clade (34). Recently, MLSA employing sequencing of four to seven housekeeping genes, such as *atpD*, *fusA*, *infB*, *gyrB*, *leuS*, *dnaA*, *pyrG*, *rplB*, *rpoB*, and *hsp60*, appeared to be the most valuable tool to identify of *E. cloacae* complex species and the most recent *Enterobacter* species (4, 118). The MLSA results were strongly validated by single-nucleotide polymorphism analysis by whole-genome sequence (4). By sequencing the *hsp60* gene, Hoffman and Roggenkamp discriminated 13 clusters (clusters I to XII and cluster XIII, which corresponds to an unstable sequence crowd) within the group (3, 55). More recently, Beyrouthy et al., using the same technique, confirmed the characterization of the two new subspecies of *E. hormaechei* obtained by complete genome sequencing and helped to complete the *Enterobacter cloacae* complex clusters (41, 208) (Table 4). Twelve clusters were attributed to existing species and more recently described species such as *E. roggenkampii*, *E. bugandensis*, and *E. hormaechei* subsp. *hoffmanii* (Table 4). The name *E. hormaechei* is sometimes given as a generic name for strains belonging to different clusters.

Recently, a German team implemented a combination of two techniques to identify the species within the *E. cloacae* complex: matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) MS coupled with real-time PCR (303). Since mass spectrometry does not allow the species to be distinguished from each other, this group requires associations to be established using real-time PCR that amplifies the *dnaJ* gene. This gene, identified by Pham et al., encodes a chaperone protein of the Hsp40 family and

TABLE 4 Distribution of clusters and species of the *Enterobacter cloacae* complex using the *hsp60* sequencing technique (163, 208)

Cluster	Species
I	*E. asburiae*
II	*E. kobei*
III	*E. hormaechei* subsp. *hoffmannii*
IV	*E. roggenkampii*
V	*E. ludwigii*
VI	*E. hormaechei* subsp. *oharae*, *E. hormaechei* subsp. *xiangfangensis*
VII	*E. hormaechei* subsp. *hormaechei*
VIII	*E. hormaechei* subsp. *steigerwaltii*
IX	*E. bugandensis*
X	*E. nimipressuralis*
XI	*E. cloacae* subsp. *cloacae*
XII	*E. cloacae* subsp. *dissolvens*
XIII	*E. cloacae* sequence crowd

is specific for the *E. cloacae* species (304). Precise identification of the species in the *E. cloacae* complex is important in the biological diagnosis of infections because species are differently implicated in human pathology (11). Phenotypic techniques can lead to misidentifications between very different species harboring different virulence potentials, such as *E. hormaechei* and *Cronobacter sakazakii*. However, a species identification error has no impact on antibiotic therapy, as the species of the *E. cloacae* complex have the same antibiotic resistance profiles.

CONCLUSION

Until now, only few virulence factors/genes have been described in *Enterobacter*. In addition, the precise regulation mechanisms and the molecular and functional properties involved in bacterial adaptation to various environmental stresses/conditions have not yet been precisely determined (10, 56, 145, 305–309).

A main biological behavior reported in the *Enterobacteriaceae* seems to be associated with its ability to evade the activity of a huge collection of antibacterial agents, including antibiotics, disinfectants, biocides, etc. For instance, *Enterobacter* possesses a versatile and sophisticated system for regulating the envelope permeability, as characterized above in clinical strains. It must be noted that the RamA operon, which is not present in *Escherichia* spp., plays a key role both directly and indirectly via the Mar operon: it can control the expression of porins and RND efflux complexes, and it can complement or link its proper regulation cascade to other global regulators such as SoxS or Rob. The advantage of this additional global regulator associated with the Mar regulon remains to be clarified in *E. aerogenes* and *E. cloacae*, where it can play the role of an enhancer ring or accelerate the bacterial adaptation to environmental stress and contribute to the prevalence of these Gram-negative bacteria in human infectious diseases.

The complete genome sequences of *E. aerogenes* and *E. cloacae* strains have illuminated the presence of resistance genes that contribute to the MDR phenotype, virulence, quorum sensing, and competition against other microorganisms (37, 120, 237, 310–313). Moreover, the whole-genome sequencing of clinical isolates collected during patient antibiotherapy in association with proteomic and functional studies has strongly illuminated the strategies developed by these important Gram-negative pathogens (237, 310). In this regard, the genus-level analysis and comparison of *Enterobacter* spp. versus *K. pneumoniae* indicates high genetic variation (average nucleotide diversity of 0.16 compared to 0.02) (314). This difference suggests that these individual species may have different mechanisms and evolutionary pressures that govern them. The regulation cascades involved during colonization, virulence, and resistance support physiological changes that ensure rapid and appropriate responses to environmental stresses, and this remarkable adaptability explains the presence of *Enterobacter* in the ESKAPE group.

The reemergence of *Enterobacter* as a worrying resistant pathogen is an important health concern, especially when the scarcity of new antibiotics active against Gram-negative bacteria is considered. Consequently, much effort is necessary to identify and dissect the molecular and genetic events that manage the *Enterobacter* adaptation and to clarify the remaining unknown aspects in the regulation cascade.

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